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NUTRITION REVIEWS

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NUTRITION REVIEWS

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STATUS OF SURVEYS FOR RADIONUCLIDES IN FOODS

This article is a condensation of a report presented to the Food and Nutrition Board of the National Academy of Sciences. It is intended to present a status summary of programs that are presently underway for the monitoring of foods as well as to indicate possible future approaches. No attempt has been made to consider all programs or programs in detail; rather the aim has been to present a broad picture of activities.

The Overall Problem: The primary objective of food surveys is to evaluate any possible effect of environmental contamination on the health and well-being of the population. This implies also the prediction of effects in relation to known or predicted levels of contamination.

Studies of environmental contamination in the past have generally fallen into the following categories: (1) surveys, (2) movement in the food chain, and (3) effects on the population.

It is to be emphasized that work in any one of these areas must be interrelated with knowledge of the other two. For example, if it were possible to set a threshold level for a given radiocontaminant in food, then the analytical methods could be simplified and a more thorough coverage of the food supply would be possible for the same expenditure of laboratory effort. It is also clear that knowledge is required from specialists in many disciplines, ranging from the soil chemist, agriculturist, and food technologist, to the medical personnel who must ultimately make the decisions as to effects of given levels of radioactivity in the human population.

Organization Responsibilities: The main organizations in the United States actively concerned with some aspect of radiocontamination of food are: Atomic Energy Commission, Department of Agriculture, Department of Defense, Fish and Wildlife Service, Food and Drug Administration, Public Health Service, United States Weather Bureau.

In the past, the Atomic Energy Commission has had the dominant role in regard to surveys of the food chain in the United States and is now carrying forward a comprehensive program. It appears, however, that the Public Health Service is to assume increasing responsibilities; this is in our tradition of separation of regulatory powers and production. There is also a trend for individual states, usually through Boards of Health, to engage in this type of effort.

In August 1959 a Presidential directive stated that the Department of Health, Education and Welfare should "intensify its radiological health efforts and have primary responsibility within the executive branch for the collation, analysis, and interpretation of data on environmental radiation levels." By executive order of the President, a Federal Radiation Council has recently been established to centralize responsibility for providing general standards and guidance to executive agencies in developing operating rules and regulations for radiological health protection.

Sources of Information: Information sources on a world-wide basis stem mainly from the following agencies of the United Nations: Food and Agriculture Organization, International Atomic Energy Agency, World Health Organization, U. N. Scientific Committee on Effects of Atomic Radiation.

The above organizations publish many specific reports. For example, the Food and Agriculture Organization will publish shortly the results of a meeting held in December

1959 on contamination in the food chain. The World Health Organization has published a similar report on the genetic effects of low levels of radiation, and a joint report of FAO and WHO on radiochemical analytical methods is now available.

The U. N. Scientific Committee acts as a clearing house for information from various nations and is thus an important source. There are two difficulties that presumably will be overcome in time: (a) raw data (as largely received by the U. N.) are of little use since there must be evaluation and interpretation; (b) since reports are submitted through government channels, there is sometimes loss of contact between the scientists doing the work and the users of the material. The U. N. Committee published an excellent summary report a year or so ago (*Report of the United Nations Scientific Committee on the Effects of Atomic Radiation. Supplement No. 17 (A/3838). New York (1958)*), and in another two or three years will probably publish another.

It should also be mentioned that agencies of other governments publish their own reports and these are numerous indeed. Interpretation is difficult because of lack of standardization of expression and methods and lack of critical evaluation in many instances.

The major sources of domestic information are the Atomic Energy Commission (*e.g., Joint Committee on Atomic Energy (U. S. Congress), Hearings on Fallout from Nuclear Weapons Tests, Vols. 1, 2, 3. May, 1959);* the National Academy of Sciences (*e.g., The Biological Effects of Atomic Radiation, Summary Reports. National Academy of Sciences-National Research Council. 1960);* and the Public Health Service (*e.g., Radiological Health Data. U. S. Public Health Service P B 161371-1. April, 1960*).

The emphasis in the domestic reports has been upon immediate presentation of raw data and presentation of individual viewpoints (persons and organizations). The data are to be had by anyone for the asking. Serious analyses of data are conspicuous by their absence, a reflection of the hesitancy of scientists in the field to devote the time and energy required to come to grips with the problem.

Sampling Programs: It may be of interest to trace briefly the development of sampling programs in this country. There appeared no need for radioactivity assays until after the start of testing of nuclear weapons. In 1947 a sampling program was started at the University of California at Los Angeles that dealt with areas relatively close to the centers of testing. Collections were made of foliage, soil, wild animals, etc., in order to get some idea of the extent of spread of radioactive contamination. In 1948 research laboratories of the Navy began to sample air. In the early 1950's there was recognition of the worldwide spread of radioactive materials with the realization that strontium 90 represented potentially the greatest general hazard. In the period 1951 to 1955 many individual sampling programs were started: each group seemed to have a major interest, *i.e.*, human bones, thyroids, milk, or soil. Most of these programs were supported by the Atomic Energy Commission and, although there was an informal liaison, there was no overall coordination, perhaps because of the feeling shared by most scientists that the problem was not or would not likely become a major one.

In 1956 a high altitude program was started because of the importance of estimating the stratospheric reservoir for prediction purposes. Shortly thereafter, there was considerable development along the lines of whole-body counting for radiocesium in man, and in large samples of feeds and foodstuffs. In 1957 the Public Health Service and the Food and Drug Administration began their programs of food analyses. The limiting factor is the skill, labor, and expense required for reliable analyses and the problem of selection of useful samples.

It may be instructive to describe briefly the procedures for sampling of milk in the United Kingdom since these represent the most intensive effort of its type. Milk is being analyzed for radionuclides of strontium, iodine, cesium, and for stable calcium and stable potassium. The country is divided into ten zones covering 200 milk depots. Special areas where one might expect higher values than normal are also being surveyed; for example, areas of high rainfall or low soil calcium. Actually 40 per cent of the milk supply of the country is being sampled and proportional bulking is used. Milk samples are taken every two weeks.

This procedure is based on the knowledge that the strontium appearing in the milk of a cow represents primarily that which the animal has eaten in the last two weeks. Thus the two-week sampling period can be used with little chance of missing any high points. This is a good example of how knowledge of metabolism can be most useful in the design of surveys.

In the United States there has been no comparable systematic sampling. In 1954 the first series was started using powdered milk from Perry, New York, and liquid milk purchased in New York City; this series was later extended to include four stations. Between 1954 and 1956 there are reports of various types of analyses representing up to 70 stations. The sampling varies from purchase of milk from the grocery store to the use of dried milk obtained at the plant. In 1957 the U. S. Public Health Service started reporting from 12 stations, and it is understood that this network is to be increased to 60. Individual states are beginning to analyze their own samples (New York and Minnesota as examples), and even private organizations (Consumer's Union) have become interested in milk analysis.

Of our food, milk has probably been the most important single item for analysis. This is because milk is produced regularly all-year-round, is convenient to handle, can be obtained so as to represent small or large areas, and does contain the most important radiocontaminants. *It must be emphasized, however, that the important parameter is the level of contamination of the total diet.* The use of milk as an indicator food does not necessarily imply that milk is the major contributor. As a matter of fact, if nuclear testing is not resumed, milk products may contribute less than 40 per cent of the radiostrontium intake while furnishing up to 80 per cent of the calcium intake. Because the dairy cow utilizes calcium in preference to strontium, it is expected in the future that the calcium of milk will be the least contaminated of all food sources of calcium.

In any sampling program for evaluation of existing environmental contamination, I would rate the following in decreasing order of importance: (1) levels in the human population, (2) levels in the total diet, (3) contribution of individual constituents of the diet, and (4) contribution of precursors of dietary constituents (air, soil, water).

For complete understanding of the system and for capabilities of prediction, items (2), (3), and (4) become as important as item (1).

A word now about the substances that should be considered in evaluation of food-chain contamination. The main stable elements of interest are calcium and potassium; such data are needed because we do not yet know whether strontium 90-calcium relationships or absolute strontium 90 levels are more useful for the purpose, and we do not understand fully the extent to which strontium behavior is governed by calcium. Similar considerations need to be taken into account in regard to cesium-potassium relationships.

The important radionuclides, of course, are those of strontium and cesium, with iodine of interest in short-term situations only, because of its short half-life. Induced activities must be considered, but so far seem to be important only as far as aquatic

food chains are concerned. Carbon 14 must be kept in mind because of possible long-term genetic effects. Fissionable materials as yet appear of secondary importance. Natural radioactive materials are of interest because of the information they can give on steady-state relationships between radioactivity in various parts of the food chain.

An Approach to the Survey Problem: As already indicated, monitoring the food supply of a nation for radiocontamination is a complex undertaking. By way of summary, there follows an outline of many of the important parameters that must be taken into account in the logical development of a survey program.

(1) *Nature of Contamination:* (a) Source (weapons, industrial or research usage); (b) Chemical form of nuclide; (c) Physical distribution (worldwide, local, stratospheric, climatic effects); (d) Time factors (single event, continuous production, relationship to growing season).

(2) *Effect of Contamination:* (a) Relationship of levels to presently accepted standards; (b) Possibility of high-risk population groups (aged, infirm, infants, fetuses, occupationally exposed); (c) Primary parameter of hazard (body concentration, local concentration, summation of exposures, genetic effects, somatic effects); (d) Threshold concepts (governs sensitivity of routine measurements needed).

(3) *Basic Sampling Decisions:* (a) Coverage required (geographical, mean levels, extreme levels); (b) Nuclides to be determined (stable and radioactive); (c) Foods to be sampled (reliability of using indicator foods), regional differences and representation, persistence of contamination, weighting for population density.

(4) *Techniques of Sampling:* (a) Ease of handling sampling; (b) Uniformity of product; (c) Uniformity of production (*e.g.*, milk compared to wheat); (d) Point in production-distribution-consumption chain to be sampled; (e) Size of sample, randomness, scope, frequency; (f) Estimate of variability; (g) Corollary data needed for interpretations (meteorological, dietary, stable nuclides, management practices).

(5) *Techniques of Assay:* (a) Type, reliability, standardization between laboratories; (b) Indirect measurements (*e.g.*, use of cesium 137 to indicate strontium-90 levels).

(6) *Reporting of Data:* (a) Description of sampling; (b) Description of assay; (c) Errors; (d) Standard units; (e) Interpretation.

Summary Statement: Until the present time surveys of the food chain in the United States for radioactive contamination have been carried out on a relatively small scale by individual laboratories and investigators. While no claim can be made that representative values are available for the population of the United States, there is enough evidence to demonstrate with reasonable probability that nowhere have levels reached presently acceptable limits.

In the course of events it may well be that closer attention should be given to the monitoring of foods (this situation could arise with or without the likelihood of actual harm from future weapons use, catastrophe, peacetime use of atomic energy, public concern over the wholesomeness of food supplies, or moral issues). It would be hoped that, if the need arises, recognized scientists in areas of nutrition and food technology would join with those of other disciplines to put the work on an increasingly sound basis. In the meantime it behooves organizations and individuals to ensure, to the degree possible, that various committees at all levels of government dealing with matters that involve the food chain, have technical representation by appropriate scientists.

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NUTRITION OF MAN IN SPACE

Regimens for feeding astronauts vary primarily with the duration of the expeditions into space. Closed cycle biological systems are required only in space travels of many months' duration.

Two years after Sputnik I, America's seven hand-picked astronauts completed a training course in how to eat under conditions of weightlessness similar to those found in space (*J. Am. Med. Assn.* **172,** *575* (*1960*)). While a passenger in a plane flying through a maneuver known as a parabolic curve, each of the astronauts was exposed to zero gravity on several occasions. Thus he became acquainted with handling, among other things, the highly concentrated liquid and semiliquid foods especially designed for him and contained in special squeeze-type bottles and tubes.

In the present state of development of manned space flight, the state of zero gravity has concerned physicians and bio-engineers more than any other problem bearing on nutrition. However, even though man has yet to orbit once around the earth, more emphasis is gradually being placed on the infinitely greater nutritional problems of space travel lasting many hours, days, weeks or even years.

C. T. Randt (*J. Am. Med. Assn.* **172,** *663* (*1960*)), in describing the impact of space exploration on biology and medicine, mentions the need for development of regenerative systems which use solar energy to provide fluid, food and gaseous requirements to support life during space travels of long duration. He foresees the need of extensive coordinated research into the nutritional requirements of a variety of organisms including man, and into the chemistry of the photosynthetic processes of closed ecologic systems. He even mentions the possibility of studying controlled hypothermia as a means of reducing metabolic requirements for food and fluid and protecting astronauts from stress.

In discussing the physiologic aspects of hypergravic and hypogravic states, J. E. Ward (*J. Am. Med. Assn.* **172,** *665* (*1960*)) comments on the problems of nausea and vomiting during weightlessness. He points to experimental observations (Ward, W. R. Hawkins and H. D. Stallings, *J. Aviation Med.* **30,** *151* (*1959*)) of the deglutition mechanism indicating that, with normal mastication of solids, swallowing of the semisolid bolus of food presents no problem. Poorly chewed solids, however, present difficulty in swallowing and may float above the palate creating a real danger of aspiration (*Nutrition Reviews* **17,** *129* (*1959*)). Incompetence of the cardiac sphincter of the stomach may be associated with regurgitation of stomach contents when pressure is imparted to the abdomen, especially if large volumes of fluid have been ingested.

Since the rate of passage of materials of varying densities through the intestinal tract is an inverse function of density (F. Hoelzel, *Am. J. Physiol.* **92,** *466* (*1930*)), food-passage time may be slightly reduced. However, in consideration of the length of the intestine and the surface tension of the semiliquid intestinal bolus (*i.e.*, the opportunity for absorption), Ward, Hawkins and Stallings feel that absorption of metabolites offers no problem.

The medical aspects of ambient radiations of extraterrestrial space, which run the gamut of the electromagnetic spectrum from radio waves through cosmic rays, have been discussed by P. A. Campbell (*J. Am. Med. Assn.* **172,** *668* (*1960*)). Although not specifically relating these radiations to the nutrition problems of space exploration, he cites both the hazards of these rays and also the usefulness of solar energy in such projects as producing oxygen and water from the soil of the moon, reconstituting

urine into usable water, and even incinerating feces into usable material. Visible light, essential for photosynthesis, provides the energy for biosynthetic gas exchange systems, which, because of weight restrictions, will probably be limited to space stations and lunar colonies. Since direct radiation of astronauts must be avoided, the use of shielding may produce problems of a logistical nature bearing on nutrition.

A comprehensive summary of the many bio-engineering problems of early manned space flight has been presented by R. W. Lawton, B. J. Smith and D. R. Ekberg (*Ann. N. Y. Acad. Sci.* **84,** *31* (*1960*)). While eliminating long term residence in space and the hazards of radiation from consideration, they mention in detail such matters as launch acceleration, re-entry deceleration, re-entry at escape velocity, landing impact on the earth, weightlessness, noise, vibration, control of the gaseous environment, the relative virtues of four-gas (oxygen, carbon dioxide, water and nitrogen) and three-gas (oxygen, carbon dioxide and water) systems, explosive and rapid decompression, temperature control, instrumentation, communications systems, biological monitoring and safety and reliability standards.

With respect to nutrition, they point out only the necessity of feeding a low-residue diet for several days preceding as well as during a space flight, and suggest that vitamin and mineral supplements for short flights may be neglected. Although nutrition per se is mentioned only briefly, it is impossible to read this enumeration of the physiologic stresses involved in early-manned space flight without recognizing that these pose problems which distinctly bear upon nutrition, especially when projected ahead to more prolonged missions in space.

In an article more directly devoted to nutritional research for the space traveler, B. Finkelstein (*J. Am. Dietet. Assn.* **36,** *313* (*1960*)) has discussed how, what and when the astronaut will eat (*see Nutrition Reviews* **18,** *100* (*1960*)). According to the author, the comparatively simple answer to "how" lies in serving liquids and semisolid foods from collapsible squeeze tubes. A pontube fitted to the end of each squeeze tube will be placed directly in the mouth and food transmitted by applying pressure to the tube. Solids in bite-size form will be removed by hand from a covered container and placed directly into the mouth. For reasons of cleanliness and sanitation, a disposable, lightweight, greaseproof, waterproof polyethylene mitten will be worn over the pressure gloves while eating. According to the author, this is necessary in order to minimize transfer of foreign particles to extremely delicate instruments.

"What" will the astronaut eat is a question the author finds less easily answered, largely because of the psychological role that food plays in a situation of stress. Sensory deprivation tests, achieved by placing subjects in totally dark (comparable to dustless space) soundproof chambers, were conducted with 50 persons for periods ranging from six to 168 hours. Evidence from these tests suggests that food in a situation of stress may be used as a tool to obtain personal satisfaction. Although caloric intakes remained within limits normal for sedentary individuals in a comfortable environment, eating patterns varied from the usual three-meal-a-day cycle to frequent snacking throughout the 24 hours.

Palatibility and acceptability were often contrary to usual tastes. Canned orange juice, usually low in acceptability ratings, rated moderate-to-high in isolation. Discrimination among foods in the same food group was impaired; all meats tasted alike and different varieties of canned fruit could not be distinguished. Breads from different flours were frequently considered similar in taste. Thus, removal of visual cues ordinarily associated with eating interferes with the taste and acceptability of food.

Manned balloon flights to altitudes of 100,000 feet have provided a short-term test situation somewhat comparable to

manned space flight. The feeding program for these balloon trials was formulated with certain criteria in mind, among them: (1) the maintenance of high efficiency on the part of the balloonist despite his extremely high activity level and the considerable stress; (2) reduction of food weight to the minimum compatible with good acceptability and adequate nutritional value; and (3) attention to weight and space limitations which restrict food storage and preclude the use of food-service equipment. In discussing the balloon trials held to date, the author seems to feel that food has assumed a significant emotional and social role. She cites the provision of food designed to alleviate rather than contribute to stress as an objective of the space-flight research program.

In the present report, she mentions several types of dietary regimens for the astronaut. The first, a pair of liquid diets each providing approximately 2500 calories and 100 g. protein, contains such items as fruit juices, coffee and chocolate flavoring, high-protein supplements, pureéd vegetables, strained meats and a concentrated ice cream mix. Preference ratings on individual items revealed the common pattern of acceptability, *i.e.*, familiar items rated high and unfamiliar laboratory concoctions low, with the latter gradually increasing in acceptability as the tests continued. The second dietary regimen (for the balloonist) contains similar ingredients but includes semiliquid chicken and beef in tubes as well as nuts and pound cake (the last two for psychological reasons). The third regimen, proposed to the National Aeronautics and Space Agency for subjects participating in Project Mercury, includes plans for preflight and in-flight feeding. The preflight diet, designed to alleviate the necessity for defecation during flight, is a high-protein, low-residue diet to be fed for 72 hours prior to take-off. For the meal immediately before take-off, a high-carbohydrate, moderate-protein, low-fat meal conducive to increased altitude tolerance is proposed. During flight, a variety of sandwiches, meats, fruit sauces and juices, chocolate drinks, cookies and candy is recommended. These foods provide 2000 calories daily with 49 per cent derived from carbohydrate, 16 per cent from protein and 35 per cent from fat. Three thousand ml. of water and a multivitamin preparation (to alleviate stress, according to the author) complete the regimen.

In answer to the final question, "when" will the astronaut be fed, the author recommends eating at two- to three-hour intervals in order to insure optimal nutrition at all times. She points out that ad libitum regulation of dietary intake according to the astronaut's desire for food and fluid might mean that an emergency could arise when the subject's state of hydration and nutrition were inadequate. Hence, she stresses the need for frequent scheduled consumption of food during unusually demanding missions.

Two preliminary evaluations dealing in part with specific approaches to the problem of nutrition in space explorations have recently been reported. R. B. Couch *et al.* (*Fed. Proc.* **19,** *13* (*1960*)) have described successful clinical trials of water-soluble, chemically-defined diets in patients with slowly progressing neoplasms. They found these satisfactory for maintenance of nitrogen balance and for repletion following depletion on a nitrogen-free diet, and superior to isonitrogenous conventional diets if 50 calories per kg. body weight per day were provided. These diets are designed from chemically defined ingredients and are available in any desired physical state applicable to short-term manned space flight.

M. E. McDowell *et al.* (*Fed. Proc.* **19,** *319* (*1960*)) have described feeding studies in humans designed to test the digestibility and toxicity of algae (*Chlorella ellipsoidea*). For a period of 33 days they fed four men increasing amounts of algae (10, 20, 50, 100, 200 and 500 g. per day) in a diet constant in calories. Their results were not unlike

those previously reported by Japanese workers (*Nutrition Reviews* **17,** *238 (1959)*). Poor acceptability, even when the algae were mixed with other food, was observed in all subjects at amounts over 100 g. daily. Nausea, fullness, abdominal aching or cramps, mild malaise and headache developed in all subjects. No diarrhea occurred, but stools became bulky, green, dry and hard. No evidences of toxicity were observed, however. These results suggest that *Chlorella ellipsoidea* may not be the organism of choice for prolonged space travel.

Concentrated synthetic diets containing 8 calories per g. are now under development (S. Miller *et al.*, *Wright Air Development Division Technical Report 60-1 (1960)*). A high calorie concentration, in addition to the adequate protein, vitamin and mineral composition of most proposed regimens, is achieved through the use of odd-carbon fatty acids. Ketosis is avoided, since from one to as many as three "three-carbon fragments" are produced per molecule. These enter pathways of carbohydrate metabolism and hence do not contribute to ketosis, thereby permitting 60 to 80 per cent of the dietary calories to be derived from fatty acids.

These recent contributions have increased by only a small measure our knowledge of space nutrition since the publication of a symposium on the subject in 1958 (*see Food Technology* **12,** *429–459 (1958)*). Many of these recent publications, as well as those in the 1958 symposium, dwell on the psychological aspects of food. While this topic may have significance in certain transportation situations, in space flight it deserves less attention than it is receiving. Astronauts are a highly selected group who can be picked for or trained to acquire an ability to tolerate any dietary regimen, provided it is nutritionally adequate and compatible with the physiology of digestion and the metabolism of the recipient. The experience with long-term feeding of liquid diets (F. Olson *et al.*, *Am. J. Clin. Nutrition* **1,** *134 (1953)*; E. H. Ahrens, Jr., *et al.*, *Ibid.* **2,** *336 (1954)*; D. M. Watkin and J. L. Steinfeld, *Fed. Proc.* **16,** *343 (1957)*) has demonstrated man's ability to adapt to an unusual diet. If the diminutive cosmos of the space vehicle demands, this adaptive ability can be exploited.

Concern, therefore, over the psychological effects of food in near-term space exploration seems unwarranted. Even in the distant future, when more prolonged space travel may be undertaken, the exploitation of man's adaptability would seem more desirable than excessive concern over his emotions as they relate to food. However, the developments in the propulsion field are so rapid that proposals are already being made (K. Ehricke, *Semiannual Meeting, Am. Soc. Mechanical Engineers, Dallas (1960)*) for a manned, nuclear-powered space vehicle, complete with bedrooms, bath, galleys, gymnasium, library and sick-bay, which will carry eight astronauts on a one and one-half year reconnaissance of Mars and/or Venus by 1970–71.

If the engineers can overcome the technical and economic problems involved in propelling a unit for food and food servicing equipment into space, they may also succeed in creating an environment in which consideration of the emotional effects of food will appear more essential than at present.

IMPORTANCE OF CHOLESTEROL IN THE HUMAN DIET

College students fed a formula diet free of fat for one week were then given fat and varying increments of cholesterol. Their serum cholesterol concentration rose rapidly until a daily intake of about 600 mg. was reached.

Now that an increased level of cholesterol in the blood is recognized as one of the factors having a positive relationship to vascular aging, more attention has been

directed toward factors regulating the concentration of cholesterol in the blood and tissues. Statistical correlation between coronary disease and the quantity of animal fats and protein has been reviewed (*Nutrition Reviews* **18,** *9* (*1960*)). Not only does the quantity of saturated fat appear to be important, but also the ratio of the amount of this type of fat to the amount of essential fatty acids seems to play a role in cholesterol metabolism (*Ibid.* **18,** *56* (*1960*)). Other nutrients may also be involved, such as the type of carbohydrate, and certainly the total caloric intake is important.

The actual quantity of cholesterol in the diet is generally considered to be of minor importance. However, a few studies have indicated to the contrary (J. M. R. Beveridge, W. F. Connell, H. L. Haust and G. A. Mayer, *Canad. J. Biochem. Physiol.* **37,** *575* (*1959*); W. E. Connor and R. E. Hodges, *J. Lab. Clin. Med.* **54,** *800* (*1959*)).

Beveridge and his group carried their studies a step further in a recent investigation (*J. Nutrition* **71,** *61* (*1960*)) employing 93 university students, of whom 75 were men and 18 were women.

For eight days these subjects were fed a formula free of fat. After this, the subjects were divided into eight groups on the basis of their serum cholesterol concentration, and were given a modified formula for another eight days containing a cholesterol-deficient butter oil (which made up 30 per cent of the calories at the expense of carbohydrate) and adequate amounts of essential vitamins. To each of the eight diets was added an increment of crystalline cholesterol ranging from zero to 1600 mg. per 950 calorie portion. This was homogenized into the formula and the subjects were given a sufficient amount of it to maintain their weight at a constant level. Total serum cholesterol was determined on days zero, four, eight 12 and 16.

During the initial period when fat was omitted from the diet, the concentration of serum cholesterol declined rapidly, the averages being: initial, 201 mg. per cent; day four, 147 mg. per cent; and day eight, 146 mg. per cent. The addition of fat and cholesterol was followed by a marked increase in the concentration of cholesterol in the blood. The authors estimated the average daily intake of cholesterol to be 13 mg. in the lowest group and almost 4500 mg. in the highest group.

Although the response of serum cholesterol concentration was rapid as the daily cholesterol intake increased up to 600 mg., there was a statistically significant rise in serum cholesterol only up to an intake of approximately 2500 mg. Greater amounts of cholesterol in the diet did not increase the cholesterolemia.

The authors concluded that the concentration of serum cholesterol in man is affected by dietary levels of this sterol, but that some moderately effective control mechanism exists in healthy young people. Presumably this mechanism is impaired in hypercholesterolemic families, in diabetics, and in some other conditions. This would explain the failure of numerous investigators to find a hypercholesterolemic effect of additional cholesterol added to the usual diet of healthy volunteers. In the present study, there was considerable leveling off of the response when more than 600 mg. of cholesterol was fed. This amount approximates the cholesterol content of the usual diet of many persons.

The demonstration of a hypercholesterolemic effect of dietary cholesterol is important to any study of diets and diseases.

This study was well planned, but had several minor defects. The short duration of observation seems undesirable, but may have been necessary. In addition, it might have been more convincing had the initial diet been one containing 30 per cent of fat devoid of cholesterol; then the addition of cholesterol alone would have been the sole variable. Also, one always wonders whether results obtained on a formula diet apply to a diet of varied foods. Nevertheless, the results are of considerable interest.

COPPER IN INFANT NUTRITION

A controlled study of prematurely born infants fed a low-copper diet failed to yield evidence of copper deficiency. Processed baby foods provide adequate amounts of copper.

In recent years, a number of clinical reports have appeared in the literature describing a syndrome characterized by microcytic hypochromic anemia, hypoproteinemia and hypocupremia (*Nutrition Reviews* **15,** *72 (1957)*). Although this syndrome is rare, its occurrence in young infants has produced a revival of interest in the role of copper in infant nutrition.

A deliberate attempt has been made to induce copper deficiency in young infants by J. F. Wilson and M. E. Lahey (*Pediatrics* **25,** *40 (1960)*). Twelve prematurely born infants (average birth weight 1200 g.) were selected. Seven were given a milk formula containing 0.07 mg. of copper per liter. This formula was fed for an average of nine weeks, the only supplement being a commercial multivitamin preparation. Records of intake were kept, and it was estimated that the daily intake of copper was about 0.015 mg. per kg. body weight per day. This level of intake is far below that usually recommended (*National Academy of Sciences-National Research Council Publication 589. Washington D. C. (1958)*), and is commensurate with that known to produce copper deficiency in swine and rats.

Four of the remaining five infants were given a milk formula containing five to eight times as much copper, while the fifth was given a supplement of copper sulfate sufficient to raise the daily intake to 45 times that of the low-copper group.

Upon dismissal from the hospital, all of the infants were given a daily supplement of iron, and foods other than milk were allowed. The observation period was continued until age six months. However, at this age biochemical data are reported for only seven of the original 12 infants.

All of the infants were carefully observed with respect to growth performance. Determinations of blood hemoglobin content, serum iron and copper, and serum proteins were made at intervals. At the end of the study the low-copper group was compared to the high-copper group.

No difference in growth performance between the two groups could be detected. With one exception, the general condition of the babies fed a low-copper diet was good. This infant developed megaloblastic anemia at age 17 weeks; serum iron and copper levels were normal, and there was a prompt response to folacin therapy. In the author's opinion, this circumstance was not due to the low-copper diet.

Both groups reached a low level of hemoglobin at about eight weeks of age, with a gradual upward trend after the fifteenth week. By six months of age the high-copper group had a higher blood hemoglobin content (11 g. per cent) than did the low-copper group (9 g. per cent), although there was no appreciable difference in serum iron content.

Serum copper levels remained low (0.04 to 0.09 mg. per cent) until about ten weeks of age; by six months of age these had risen to the range of 0.1 to 0.2 mg. per cent. Infants receiving the low-copper formula had, on the average, a slightly higher serum copper level than did the others. In both groups, there was a good correlation between serum copper and alpha-2-globulin levels. Hypoalbuminemia did not occur.

When all of the observational data are considered, it is apparent that varying the dietary intake of copper was without effect during the first two months of life. The babies fed the low-copper diet appeared normal in every respect. By six months of age, however, this group had a somewhat

lower hemoglobin level, though hypocupremia was not present, nor were other manifestations of copper deficiency.

This reported failure to provoke copper deficiency on a diet known to produce deficiency in animals raises certain interesting points. The first of these is growth rate. The authors deliberately selected prematurely born infants for their study, since growth is rapid in such infants. However, human infants grow much more slowly than do either rats or pigs if growth is portrayed in terms of the percentage or relative growth rate. Indeed, most young mammals grow at a much faster rate than the human. It could be reasonably anticipated, therefore, that the dietary requirements of most nutrients on a daily per kg. basis would be less for the human infant.

Thus the question as to what would have happened had an even lower copper diet been fed or had the present dietary regimen been extended, is still unanswered.

The diet offered to the infants in this study after two months of age is not specified (nor, for that matter, is analysis of the multivitamin preparation available), and no estimate of the copper intake during this period is provided by the authors. In this connection, a recent report by G. Hughes, V. J. Kelly, and R. A. Stewart (*Pediatrics* **25,** *477* (*1960*)) is of importance. These authors assayed some 2600 samples of 86 different processed baby foods for copper content. The foods in question, although prepared by one manufacturer, were processed in various parts of the country.

Baby cereals and beef liver are the richest source of copper in this group of foods, with average values in the range of 0.3 to 2.6 mg. copper per 100 g. (as taken from the container). Other meat products and egg yolk contained an average of 0.07 to 0.18 mg. per 100 g., mixed vegetable and meat soups 0.06 to 0.17 mg., vegetables 0.08 to 0.16 mg., fruits 0.06 to 0.13 mg., and desserts 0.05 to 0.08 mg. per 100 g. There were no significant differences ascribable to locality of packing or to the type of container (tin, glass) used.

The authors conclude that the copper intake for infants consuming an average amount and variety of processed baby foods would be at least 0.05 mg. copper per kg. body weight per day. They also point out that a diet consisting of milk of low-copper content supplemented only by foods having the lower copper content would provide only two-thirds of this amount.

While Wilson and Lahey's study does not establish the minimum requirement of copper for young infants, it does document the fact that they can thrive, for short periods at least, on intakes well below those currently recommended. How long they would have continued to thrive on such a diet is a most important question, for the majority of infants reported in the clinical literature as suffering from hypocupremia have subsisted largely on milk for periods of six to 12 months or longer. The analyses reported by Hughes, Kelly, and Stewart indicate that Wilson and Lahey's subjects conceivably could have received adequate amounts of copper from supplemental baby foods in the latter period of the study.

CARDIAC DISEASES IN THREE RACIAL GROUPS IN SOUTH AFRICA

In Capetown, the Bantus have less coronary disease but more rheumatic heart disease and pericarditis. European residents have a reversed incidence. Dietary factors may contribute to these findings.

Although much has been written about the incidence of coronary vascular diseases in a number of different countries, the relative roles of hereditary environment, diet,

occupation, and emotional stress are difficult to assess. In the United States, H. I. Russek (*J. Am. Med. Assn.* **171,** *503 (1959)*) found that the emotional response to insecurity, frustrations and restlessness seems to be a more important factor than diet, the use of tobacco, heredity or obesity. In Japan and Thailand, O. J. Pollak (*Am. J. Clin. Nutrition* **7,** *502 (1959)*) found the incidence of coronary disease to be the inverse of the amount of animal fats eaten by the populace. Despite these exceptions, most students of nutrition agree that dietary fats of a saturated nature if not balanced by an adequate intake of certain unsaturated fatty acids (linoleic acid equivalent) may contribute to hypercholesterolemia and to vascular disease.

In Capetown, South Africa, three groups of people differing in ethnic origin, social-economic status, and dietary customs live in close proximity. The Bantus are a tribe of middle African origin who live in relative poverty and eat a diet containing few animal fats and modest quantities of protein. The "Cape Coloreds" are a mixture of Asiatic, European and African peoples who occupy an intermediate position in the social economic structure and eat a diet containing more fats and proteins. The white population is of European origin. Their diet contains a great deal more of these expensive foods.

Quite naturally this situation has attracted students of heart disease to conduct surveys of several types (B. Bronte-Stewart, A. Keys, and J. F. Brock, *Lancet* **II,** *1103 (1955)*; Bronte-Stewart, *Brit. Med. Bull.* **14,** *243 (1958)*). Although these studies initially suggested that the Bantus had a remarkably low incidence of coronary vascular disease, closer scrutiny of the data discloses that these people have types of heart disease other than atherosclerotic.

In a similar study of the three racial groups in Capetown, V. Schrire has compared the Bantus, the "Cape Coloreds", and the whites (*Am. Heart J.* **59,** *835 (1960)*). The author chose data from hospital records of over 1300 patients of all ages, both sexes, and all three racial groups. These patients had been hospitalized in the teaching beds of two hospitals (898 beds) over a period of five consecutive years. They had had electrocardiographic tracings because of suspected heart disease and, in nearly all instances, the tracings were examined by the author. Fifty-seven per cent of the records were from whites, 37 per cent from "Cape Coloreds", and 6 per cent from Bantus.

The diagnoses were tabulated according to standard classifications and by racial origin. As one might anticipate, the white people had a much higher incidence of coronary vascular disease (26 per cent) than did the "Cape Coloreds" (13 per cent) or the Bantus (1 per cent). Interestingly enough, the incidence of hypertension was similar for all three groups (46, 51 and 35 per cent, respectively.) However, rheumatic heart disease occurred more commonly in the "Cape Coloreds" (25 per cent) and in the Bantus (20 per cent) than in the whites (12 per cent).

Beri-beri and "cardiopathy presumably of dietary origin" occurred in 0.5 per cent of the white people, in 1.5 per cent of the "Cape Coloreds" and in five per cent of the Bantus. Most of the patients of all three races with this diagnosis were considered to be alcoholic. Another strange finding was an incidence of 15 per cent of pericarditis in the Bantus compared with only 2 per cent in the "Cape Coloreds" and 0.5 per cent in the whites.

These findings would suggest that the white population, by virtue of their diet or their heredity or their social-economic status, had relative protection against rheumatic fever, nutritional heart disease and pericarditis, but had an increased susceptibility to coronary vascular disease. Conversely, the Bantus, presumably for

the same reasons, were more susceptible to rheumatic fever, pericarditis, and nutritional heart disease, but were relatively immune to atherosclerosis of the coronary vessels. An intermediate position was held by the "Cape Coloreds", except for a modest increase in the incidence of hypertensive disease.

A. R. P. Walker and H. Grusin (*Am. J. Clin. Nutrition* **7**, *264* (*1959*)) compared the incidence of coronary disease to that of cerebral vascular disease in the Bantus of Johannesburg, South Africa. They concluded that, although accurate statistics were difficult to obtain, there seemed to be a higher incidence of cerebral than of coronary vascular disease in these people. They suggested that studies of salt intake in the diet and of the incidence of essential hypertension might be revealing.

The present report by Schrire deals in part with hypertension. It was found that its occurence in the Bantus was slightly less than in the other groups. Furthermore, its reasonably even distribution among all three racial groups was comparable to the 21 per cent of all hospitalized patients in India and 26 per cent of all hospitalized patients in the United States. Thus these data suggest that neither diet nor social-economic status nor racial origin plays a critical role in the etiology of essential hypertension.

This study, although derived from crude data, provides information pertinent to the interpretation of other reports. It suggests that as the social-economic status increases and the diet becomes more abundantly supplied with foods of animal origin, the incidence of pericarditis and rheumatic fever declines in a reciprocal relation to that of coronary atherosclerosis. One might compare these disorders to opposite ends of a teeter-totter in which hypertensive disease serves as a fulcrum. These speculations may aid in formulating the designs of additional studies which will aid in the selection of diets compatible with a low incidence of all types of heart disease.

NEW ANTIMETABOLITES OF VITAMIN B_{12}

In a search for antimetabolites of vitamin B_{12}, 15 promising compounds were found. These included benzimidazoles, diazobenzimidazoles, purines, pyrimidines, pteridines and niacin derivatives.

Perhaps one of the most challenging forms of medical investigation involves the concept of antimetabolites. In recent years we have observed the dramatic effects of a few, such as the sulfonamides, the coumarin drugs, the anti-folic acids and the antibiotics. D. W. Woolley has done much to turn the imagination of investigators toward studies of new antimetabolites (*A Study of Antin etabolites. John Wiley & Sons, Inc., New York* (*1952*)). He discussed a compound, 1,2-dimethyl-4,5-diaminobenzene, which he considered to be a precursor of two important vitamins, riboflavin and vitamin B_{12}. The latter has been studied intensively because of its unique role in the processes of hematopoiesis and in the metabolism of the central nervous system. Evidence suggests that it also is vital to the cellular functions of the kidney, the muscles and other tissues. Although dimethyl-diaminobenzene was shown to have slight anti-vitamin B_{12} activity, there has been no antimetabolite which could effectively inhibit all of the actions of this vitamin.

Recently M. Bodian (*Pediat. Clin. N. Am.* **6**, *449* (*1959*)) reported that large doses of vitamin B_{12} caused regression of neuro-

blastomas. Another report by Woolley linked neoplastic disease with vitamin B_{12} (*Proc. Nat. Acad. Sci.* **39,** *6 (1953)*). He found that spontaneous mammary tumors of mice seemed to be synthesizing vitamin B_{12}. L. Smith (*Nature* **181,** *307 (1958)*) studied some analogues of vitamin B_{12}, and another group employed 2-ethyl-2,3-naphthimidazole-4,9-dione to suppress the growth of mammary carcinoma of mice.

These considerations led to a study by G. M. Timmis and S. S. Epstein (*Nature* **184,** *1383 (1959)*), in which they employed approximately 100 analogues of vitamin B_{12}. The test organism was *Euglena gracilis*, a flagellated green alga whose growth can be measured readily (S. H. Hutner *et al*, *Proc. Soc. Exp. Biol. Med.* **70,** *118 (1949)*). Those chemical compounds, which in concentration of 500 μg per ml. induced 50 per cent inhibition of this organism, were selected for further study. The test systems compared growth of *E. gracilis* in the presence of 0, 10, 100 and 1000 $\mu\mu$g. with varying quantities of the antagonists added. Results were expressed as that quantity of an antagonist needed to induce 50 per cent inhibition of growth at each concentration of vitamin B_{12}.

The compounds included six structural types: benzimidazoles, diazobenzimidazoles, purines, pyrimidines, pteridines and niacin derivatives. In each of these groups, inhibition of growth in the presence of 10 $\mu\mu$g. of vitamin B_{12} was accomplished by addition of 50 to 200 μg. of the antagonist. This would indicate that these compounds are sufficiently active to justify animal studies.

Of particular interest is the finding that derivatives of pteridines and of niacin have anti-vitamin B_{12} activity. Unlike the benzimidazoles and the purines, they are not analogous in structure to any part of the vitamin B_{12} molecule.

Although 6-mercaptopurine, used in therapy of acute leukemias, has an inhibitory effect upon the synthesis of nucleic acids and proteins, the authors considered that it may exert some antivitamin B_{12} action also since it was a potent inhibitor in the present study.

Development of new and potent inhibitors of vitamin B_{12} undoubtedly will prove to be important in the study of neoplastic diseases as well as of normal hematopoiesis. This report should stimulate many investigative efforts.

EFFECTS OF FORCE FEEDING

Rats eating ad libitum have less body fat than those force fed the same amount of feed. Force feeding produces a threefold increase in activity of enzymes involved in lipogenesis.

During the past few years a number of investigators have reported that when animals are force fed they show altered physiological effects. A large amount of work in this area has been done by C. Cohn and associates, who found that when rats are tube fed they deposit more fat in their bodies than animals eating ad libitum. This was true even though the force-fed animals received only enough food to produce a rate of gain in body weight equal to that of the ad libitum controls.

Their attention was directed to the above problem by the observation that adrenalectomized animals deposited much more body fat when force fed than did the control animals. The work with adrenalectomized rats was stopped when it was observed that normal animals behaved in the same way (C. Cohn, D. Joseph and E. Shrago, *Metabolism* **6,** *381 (1957)*). Normal rats force fed enough food to produce the same weight gain over a two-week period as animals normally eating had approximately twice as much body fat as the controls.

In order to determine whether the in-

creased body fat in the force-fed animals was caused by their receiving all of their food twice a day, the investigators fed three separate groups of animals by stomach tube in 2, 3 or 4 portions, respectively, during the day. During the two-week trial period the average weight gain for the animals in these groups ranged from 61 to 64 g. The body fat content, however, was the same in all three groups.

In another trial (Cohn *et al.*, *loc. cit.*), rats were trained to eat their daily quota of food within a two-hour period (a semipurified diet suspended in water). However, these animals had the same percentage of body fat as the controls with access at all times to the same diet except in the dry state.

Finally, to determine whether the difference in the consistency of the diet had any effect on the body composition of the ad libitum-fed rats, a group of ten animals was fed the dry ration and another group of ten received the same diet mixed with water. These rats were compared with a group of ten receiving the diet mixed with water but force-fed in amounts which produced the same rate of gain as that of the group receiving the solid diet on an ad libitum basis. The consistency of the diet had no effect on the percentage of body fat in the ad libitum groups. However, the force-fed group had a larger amount of body fat than the average for all ad libitum-fed rats (17 *vs.* 12 per cent).

Since the tube-fed animals were depositing more fat, it was obvious that they were metabolizing fewer calories than the ad libitum-fed controls. For this reason, Cohn and associates (*Endocrinology* **62,** *251 (1958)*) examined a number of parameters of thyroid function. All of the animals, as in the previous studies, weighed between 150 to 160 g. at the start of the experiment. The force-fed animals were adapted to the liquid diet over a period of one week in order to avoid "food shock." They were fed twice daily in the early morning and late afternoon. After two weeks of such feeding, the ad libitum controls had an average body weight of 230 g. while the force-fed rats averaged 227 g. At that time, the thyroid glands of the ad libitum controls took up 7.94 per cent of the administered I^{131} in 24 hours, whereas the thyroids of the force-fed animals took up only 1.71 per cent.

As a further measure of thyroid activity, the amount of administered I^{131} bound to the plasma protein (PBI) was determined. The ad libitum-fed rats incorporated one and one-half times as much I^{131} into PBI as the force-fed rats. The concentration of injected I^{131} iodide in 1 mg. of thyroid tissue over the concentration in 1 ml. of serum (T/S ratio) was the same for both the force-fed and ad libitum-fed animals. According to the authors, these data suggested that force feeding produces hypothyroidism, which is secondary to a decreased production or release of thyroid-stimulating hormone from the anterior pituitary gland.

In another set of experiments, Cohn and Joseph (*Am. J. Physiol.* **196,** *965 (1959)*) used the paired-feeding technique; the rats on the ad libitum regimen and those that were force fed received the same amount of diet. The latter were fed by stomach tube twice each day. After two weeks of such feeding, the animals were sacrificed and the tail and hair removed and discarded. Carcass analyses indicated that, although both groups of rats showed the same increase in body weight (58 and 56 g. respectively), the force-fed animals had 26 g. of fat in their bodies, whereas the ad libitum-fed animals had 19 g.

The rapid accumulation of fat by the force-fed rats was sharply demonstrated upon comparison with animals that had been sacrificed prior to the actual start of the experiment. (All animals had been maintained on their respective feeding regimens for one preliminary week.) The force-fed rats sacrificed at that time had an average of only 13 g. of fat in their bodies, and the ad libitum-fed rats had 15 g. The increase in body fat during the actual experiment

was, therefore, three times as great in the force-fed rats as in the controls.

Calculations by the investigators showed that 24 per cent of the tissue laid down by the force-fed animals was fat, whereas fat accounted for only 7 per cent of the tissue acquired by the rats fed ad libitum.

The bodies of the above animals were divided into skin, viscera and residual carcass, and each of these was analyzed separately. The results indicated that the skin of the force-fed rats showed the greatest relative increase in fat (eight times greater than in the ad libitum controls). There was relatively little difference in the rate of accumulation of fat in the viscera, while the fat increase in the residual carcasses of the force-fed animals was twice that of rats fed ad libitum.

In order to determine whether the more rapid presentation of food to the body, as in force feeding, has any effect on metabolic pathways, Cohn and Joseph (*Am. J. Physiol.* **197,** *1347 (1959)*) studied the hexosemonophosphate shunt activity in the livers and epididymal fat pads of the force-fed and control animals. The glucose-6-phosphate dehydrogenase activity was two to three times greater in both the liver and adipose tissue of the tube-fed animals than in the tissues of the ad libitum-fed controls. The investigators pointed out that the shunt is an important source of reduced TPN and that fatty acid synthesis is dependent, among other things, upon the generation of the reduced nucleotide. They conclude from this work that forced feeding of meals is associated with adaptive enzyme changes favoring lipogenesis.

The authors suggest that the rate of ingestion of foodstuffs influences the intermediary metabolic pathways to which the food is subjected. As evidence for this, they refer to the work of N. Werthessen (*Am. J. Physiol.* **120,** *458 (1937)*) and J. Tepperman, J. R. Brobeck and C. N. H. Long (*Yale J. Biol. Med.* **15,** *855 (1942)*), who observed lower respiratory quotients in rats trained to eat their entire day's ration during a restricted period of time than in the ad libitum-fed controls. Similar differences in the respiratory quotient of liver slices from animals trained to eat their food in a short period of time were observed by V. C. Dickerson, Tepperman and Long (*Yale J. Biol. Med.* **15,** *875 (1942)*). The above results were interpreted as indicating excessive deposition of fat in animals adapted to eating their food within a short period. This adaptation probably stems from the development of "alternate and perhaps more 'efficient' pathways."

To explore the difference in rate of food ingestion on the development of a metabolic disease, Cohn, R. Pick and L. N. Katz (*Circulation* **20,** *969 (1959)*) permitted a group of chicks access to food for an hour in the morning and for another hour in the afternoon. The other group was permitted the amount of feed consumed by the first group but ate at will. The diet contained 0.5 per cent cholesterol, 5 per cent cottonseed oil and 20 per cent protein. After five weeks, the chicks eating only twice a day had an average serum cholesterol of 584 mg. per cent while that of the other group was 294. The thoracic aortas of the chicks eating twice a day had a higher incidence of atherosclerosis (100 *vs.* 44 per cent), which was also more severe. Microscopic examination showed that 14.6 per cent of the coronary vessels in "meal eaters" (receiving food once or twice a day) had atherosclerosis while only 2.2 per cent of the vessels in the nibblers were so affected.

In another experiment, half of a group of chicks that had developed elevated levels of blood cholesterol were fed the non-cholesterol ration for one hour each day. These chicks cleared their coronary arteries only one-third as rapidly as chicks eating the non-cholesterol diet throughout the day.

The studies of Cohn and associates are intriguing. It must be pointed out, however, that in their earlier work (*Metabolism* **6,** *381 (1957)*) they reported that even when the

day's food quota was given in four equal stomach tubings, the rats showed the same accumulation of body fat as did those receiving all of their food once a day by stomach tube. Cohn and Joseph explain this apparently discordant observation by suggesting that they "did not feed frequently enough nor spread the feedings over a sufficient number of hours daily" (*Ibid.* **9,** *492* (*1960*)). However, of greater consequence was their report (*Ibid.* **6,** *381* (*1957*)) that when rats were trained to eat their day's food in an hour, their body fat content was the same as that of the ad libitum-fed controls. If credence is to be given the suggestion that the rate of ingestion of food influences metabolic pathways, then the above discrepancies should be clarified by experimental observations.

Another criticism of the work of Cohn and associates is that within a two-week experimental period changes may be seen which on further observation disappear. It is entirely possible that here, as in many other situations, a two-week period is too short for establishing an equilibrium condition.

One factor discussed by Cohn and Joseph (*Metabolism* **9,** *492* (*1960*)) is that the forced-fed rat "conserved more calories, which were retained as fat, than his nibbling partner eating ad libitum." Actually, the differences in the caloric balances of the two groups of rats may be relatively small, about 5 Calories per rat per day. The latter was calculated from the composition of the body weight gained during the two-week experimental period (*Am. J. Physiol.* **196,** *965* (*1959*)). If possible, a caloric balance should be carried out to clarify this point.

Finally, it should be pointed out that when rats had access to a cholesterol-containing diet for three hours each day, they had cholesterol levels of 61 mg. per 100 ml. of serum, while animals with access to the same diet 24 hours a day had cholesterol levels of 85 mg. (R. Okey, G. Scheier and M. Reed, *J. Am. Diet. Assn.* **36,** *441* (*1960*)). The animals used in this later work were started on the experiment at weaning and maintained for seven weeks. The preceding observation makes it all the more imperative to clear up the questions that have been directed at the evidence used in building a hypothesis as to the effect of meal-eating as contrasted to nibbling.

Regardless of the above criticism, these studies merit consideration by both animal experimentalists and those interested in human nutrition since they suggest that by altering the eating pattern of an animal one may be able to alter a variety of physiological functions or speed up the development of an abnormality (atherosclerosis). Thus science may be provided with a new tool comparable to the "thyroid stress" used so frequently in a variety of studies.

Cohn's work also suggests that pair-fed rats and those permitted to eat ad libitum may not be as comparable as investigators in the past have thought them to be and that caution should thus be exercised in interpreting results of paired-feeding experiments, especially those lasting for only a few weeks.

On the basis of the above work, it would appear justifiable to record the eating pattern of human beings when dietary surveys are included in a medical or biochemical study.

NUTRITIONAL MUSCULAR DYSTROPHY

The development of muscular dystrophy in calves on a low vitamin E diet was intensified by feeding cod-liver oil. The resulting low serum magnesium levels may have accentuated the muscle changes.

Although muscular dystrophy develops in many different species when fed diets deficient in alpha-tocopherol, the severity of the lesions and the time required for their

production in the different species varies a great deal. D. C. Maplesden, J. D. Harvey and H. D. Branion (*Canad. Vet. J.* **1,** *10* (*1960*)) formulated a diet which produced muscular dystrophy in calves, but the time required for the development of the dystrophic lesions was extensive. Calves have been shown by other investigators to be quite resistant to muscular dystrophy when compared to other species such as guinea pigs, sheep and goats (G. K. Davis and L. A. Maynard, *J. Dairy Sci.* **21,** *143* (*1938*)). Thus an extended feeding period may be required to produce muscular dystrophy in calves on a diet low in unsaturated fatty acids and the tocopherols.

However, K. L. Blaxter, W. A. Wood and A. M. MacDonald (*Brit. J. Nutrition* **7,** *34* (*1953*)) demonstrated that cod-liver oil added to the diet of the calf would nullify the action of DL-alpha-tocopherol in the diet, and this effect of the unsaturated fatty acids in cod-liver oil apparently extends to the tocopherols already present in the animal body.

The report by K. Schwarz and C. M. Foltz (*J. Am. Chem. Soc.* **78,** *3292* (*1957*); see also *Nutrition Reviews* **18,** *193* (*1960*)) that selenium was an integral part of Factor 3 and that inorganic selenium salts were remarkably effective in protecting against dietary necrotic liver degeneration in rats has rapidly led to studies with other species. Selenium was reported by O. H. Muth *et al.* (*Science* **128,** *1090* (*1958*)) to prevent muscular dystrophy in lambs just as effectively as vitamin E, but the changes in tissue mineral element content with muscular dystrophy reported by many investigators suggest the condition is not a simple vitamin E deficiency.

In an effort to differentiate the action of selenium and alpha-tocopherol as nutrients which prevent muscular dystrophy, Maplesden and J. K. Loosli (*J. Dairy Sci.* **43,** *645* (*1960*)) made use of the diet described by Maplesden, Harvey and Branion (*loc. cit.*), but intensified the production of a tocopherol deficiency by adding cod-liver oil. While no gross evidences of muscular dystrophy appeared (with one possible exception), all of the calves not supplemented with alpha-tocopherol showed low plasma tocopherol levels, averaging 108 μg. of total tocopherol per 100 ml. of plasma, as compared to 639 μg. found in calves receiving supplementary D-alpha-tocopherol acetate.

All of the calves maintained on the alpha-tocopherol-deficient diet plus cod-liver oil for more than 90 days showed, upon histological examination, Zenker's degeneration of the skeletal muscles and degeneration of the Purkinje fibers of the heart. On the other hand, none of the animals receiving tocopherol had any evidence of muscular dystrophy or heart lesions. Interestingly enough, the addition of 1 p.p.m. of selenium to the diet (which already contained 0.3 p.p.m. of selenium) had no effect upon the development of the muscular dystrophy.

It was quite evident that the addition of cod-liver oil produced much more extensive lesions than observed by Maplesden, Harvey and Branion (*loc. cit*), but the calves continued to eat and grow normally. The development of muscular dystrophy without gross clinical symptoms makes it readily understandable how sudden exercise in the spring could aggravate or precipitate Zenker's degeneration.

It was interesting that in the experiments of Maplesden and Loosli the blood magnesium levels dropped in those calves receiving cod-liver oil. Since B. A. Dehority *et al.* (*J. Animal Sci.* **17,** *1183* (*1958*)) had observed that additions of cod-liver oil to the diet of calves caused a decrease in blood serum magnesium and since the basal diet used by Maplesden and Loosli contained 20.2 mg. of magnesium per 100 ml. of liquid diet (an adequate level), it may be suggested that the cod-liver oil was interefering with magnesium utilization, possibly through the formation of magnesium soaps.

Since Blaxter, J. A. F. Rook and Mac-

Donald (*J. Comp. Path. Therap.* **64,** *157 (1954)*) did not observe any degeneration of the Purkinje fibers in calves that developed a hypomagnesemic tetany on a magnesium-deficient diet fortified with tocopherol, Maplesden and Loosli suggest that the intensification of the Zenker's degeneration and the changes in the Purkinje fibers may represent a synergistic effect brought on by the low levels of tocopherol and magnesium. Certainly the possibility that the unsaturated fatty acids of cod-liver oil not only interfere with vitamin E utilization but also with mineral element absorption is an interesting one.

The ineffectiveness of selenium in the experiments of Maplesden and Loosli emphasizes the need for basic information on the mechanism whereby dietary constituents are utilized. The complexity of the factors which interfere with absorption and utilization suggests that this field of research, especially respecting the functions of trace elements, has hardly been scratched.

FATTY LIVER INDUCTION BY OROTIC ACID

The orotic acid fatty liver is prevented by a natural diet or by adenine and must be an indirect result of deranged nucleotide metabolism.

Orotic acid (6-carboxy-2,4-dihydroxypyrimidine) has been shown to be an intermediate in pyrimidine nucleotide synthesis in both microorganisms and higher animals (I. Lieberman and A. Kornberg, *J. Biol. Chem.* **207,** *911 (1954)*; Lieberman, Kornberg and E. Simms, *Ibid.* **215,** *403 (1955)*). It is apparently formed from carbamyl aspartate and converted to orotidine-5-phosphate and to uridine-5-phosphate, thus entering intimately into nucleotide synthesis and metabolism. It is probably formed in small amounts in many tissues and is a minor constituent of many dietary substances such as milk.

It was surprising, therefore, to find that when rats were weaned on purified diets containing 1 per cent or less of orotic acid, they developed fatty livers (S. B. Standerfer and P. Handler, *Proc. Soc. Exp. Biol. Med.* **90,** *270 (1955)*). Moreover, the mechanism of fatty liver production was not apparent since the usual lipotropic agents, such as choline, methionine, folacin or cobalamine did not prevent the fat accumulation.

In order to clarify these observations, R. E. Handschumacher and co-workers (*Proc. Nat. Acad. Sci.* **46,** *178 1960*)) studied the production of fatty livers by orotic acid and its relationship to other nutritional and biochemical factors. Weanling rats were given either a commercial dog chow diet or a purified diet containing 18 per cent casein, 72.8 per cent sucrose, 2 per cent corn oil, 2.2 per cent vitamin mixture and 5.0 per cent salts, with additional choline chloride at 0.3 per cent of the total. When 1 per cent orotic acid was included in the purified diet, fatty livers developed maximally within seven days. In all other respects, these rats appeared to be normal except for a somewhat lower growth rate.

Since 1 per cent orotic acid in the dog chow diet did not induce fatty livers, a search was made for factors which might be responsible for a possible protective agent. When it was found that, of many substances tried, only 0.25 per cent of adenine sulfate or compounds containing adenine would prevent or reverse the orotic acid fatty livers, it seemed evident that nucleotide metabolism was involved in some way.

Several experimental results appeared to confirm this notion. The amounts of ribonucleic acid in livers of animals fed either the purified orotic-acid-supplemented diet plus adenine or the dog chow diet were

greater than in those of animals fed the purified diet alone or this diet plus only orotic acid. When the rate of formation of uridylic acid from orotic acid was measured (by evolution of $C^{14}O^2$ from orotic acid-7-C^{14}) in liver slices, a lower rate was found for livers of animals fed orotic acid and an increase for those with supplemental adenine or fed the dog chow. Moreover, conversion of adenine to adenine nucleotides was depressed in the 106,000 × g. supernatant solutions of livers from rats fed 1 per cent orotic acid, but was, however, not restored by adenine or dog chow even though the fat accumulation was prevented. It was also noted that degradation of orotic acid-2-C^{14} was markedly depressed in liver slices of rats with dietary orotic acid, but was then greatly enhanced by removal of the orotic acid or inclusion of adenine.

From these results, the authors suggest that the fatty livers are caused by a reversible metabolic imbalance brought about by excess orotic acid even though this substance is a normal metabolic intermediate. Prevention of this effect by adenine suggests that the action of orotic acid lies in stimulation of additional synthesis of nucleic acids, thus reducing the pool of nucleotides and possibly bringing about shortages of coenzymes of certain purines and pyrimidines, which are not replaced in the purified diet. In particular, the adenine nucleotides appear to be limiting in this case, and it is possible that an adenine coenzyme is involved in some stage of the removal of lipid from the liver.

The authors also call attention to a report (L. E. Hallanger, J. W. Laakso and M. O. Schultze, *J. Biol. Chem.* **202,** *83 (1953)*) that, although human milk contains insignificant amounts of orotic acid, cows' milk may contain it to the extent of 0.2 per cent of the solids. Although it is doubtful that this amount would lead to any abnormalities in liver function, especially with the additional nutrients of the milk, it is a possibility which may deserve some consideration.

SOME FUNCTIONS OF VITAMIN E

Premature infants and all newborns have low serum levels of tocopherol without incurring hemolytic anemia. The biochemical effects of vitamin E deficiency may be due to peroxidation of lipids of cellular mitochondria.

Although the factor known as vitamin E has been studied for 40 years, the mechanism of its action and indeed its effects on man remain poorly understood. The characteristics of vitamin E deficiency in animals have been described repeatedly. These include retardation of growth of the young, infertility of adults, and the development of myopathies of both skeletal and cardiac muscle. In addition, encephalomalacia, degeneration of the kidneys, and several other peculiar syndromes have been observed (*Nutrition Reviews* **17,** *60, 154 (1959)*). Animals with muscular dystrophy may have ceroid deposits in their muscular tissue and they excrete abnormal quantities of creatine in their urine.

Muscle from deficient animals has an increased rate of oxygen utilization (O. B. Houchin and H. A. Mattill, *J. Biol. Chem.* **146,** *309, 313 (1942)*). This observation led to the discovery that vitamin E has an antioxidative effect. The erythrocytes of deficient animals can be hemolized more readily than normal by dilute solutions of hydrogen peroxide (C. S. Rose and P. György, *Am. J. Physiol.* **168,** *414 (1952)*).

If human deficiency of vitamin E is to occur spontaneously it must happen in persons who have some absorptive defect

or in those who are fed an unusual diet, since the vitamin is both widely distributed and readily absorbed. These are precisely the circumstances present in premature infants, whose absorptive capacities are incompletely developed and who are usually given a diet composed primarily of dilute skimmed milk and carbohydrate.

For this reason, R. B. Goldbloom (*Canad. Med. Assn. J.* **82,** *1114 (1960)*) studied the concentration of tocopherols in the serum of infants and children in comparison with that of adults. He also measured erythrocyte survival of chromium-51 tagged cells in laboratory animals and in one subject in an attempt to find a hemolytic process. He measured the tocopherol content of serum from healthy adults, normal premature infants, full-term infants less than seven days old, healthy children between the ages of two and 13 years, children with biliary atresia and patients with fibrocystic disease of the pancreas.

Normal adults had concentrations consistently above 0.5 mg. per cent with a mean value of 0.77 mg. per cent, and normal children had similar values. Healthy infants, permature infants and those with biliary atresia had values of about 0.2 mg. per cent. Patients with fibrocystic pancreatic disease had the lowest average values (0.11 mg. per cent).

Erythrocyte survival studies in vitamin E-deficient rats and in a two-year-old boy with fibrocystic disease of the pancreas (tocopherol level of 0.02-0.26 mg. per cent) were quite normal.

These findings led the author to consider that premature infants and children or adults with absorptive defects might benefit from the addition of tocopherol to their diet. The author is conducting an additional study of the effects of supplementing the diet of premature infants, the results of which should be of considerable interest.

It seems particularly helpful to learn that deficiency of vitamin E did not cause erythrocytic hemolysis despite the fact that these red cells are more susceptible to hydrogen peroxide. This observation would have been of much greater significance had the author determined hydrogen peroxide fragility of the blood specimens.

Aside from the antioxidant effect of vitamin E, very little is known regarding its mechanism of action. In a separate article, H. Zalkin and A. L. Tappel (*Arch. Biochem. Biophys.* **88,** *113 (1960)*) reported evidence for postulating that this vitamin acts by preventing lipid peroxidation of mitochondria. They fed a vitamin E-deficient diet to white rabbits until muscular weakness developed. Then they sacrificed the animals along with healthy controls and studied the thiobarbituric acid reaction of their tissues. (This reaction measures malonaldehyde, which is formed by lipid peroxidation, and can be used to measure peroxidation of tissues and of mitochondria.) In deficient animals they found significantly more thiobarbituric acid reactant in the kidneys and in the liver than in healthy animals.

Their study further sought to determine whether the alterations of the mitochondria might affect oxidative phosphorylation. It was found that this function was impaired with regard to phosphorylation of succinate but not with regard to beta-hydroxybutyrate or alpha-ketoglutarate.

The demand for vitamin E can be met by supplying animals with other antioxidants (*Nutrition Reviews* **17,** *174 (1959)*), and yet not all of the actions of this vitamin are duplicated by such compounds (*Ibid.* **17,** *340 (1959)*; **18,** *193 (1960)*). Zalkin and Tappel discussed the comparative effects of vitamin E deficiency and those of ionizing radiation. At least part of the toxicity of such radiation may depend upon production of lipid peroxides (V. J. Horgan *et al.*, *Biochem. J.* **67,** *551 (1957)*). Since lipid peroxidation may result in such free radicals as lipid peroxyls, lipid oxyls, lipid hydroxyls, and others resulting from cleavage of unsaturated fatty acids, these products might

be expected to react with and inactivate a variety of compounds including enzymes and vitamins (Tappel, *Arch. Biochem. Biophys.* **50,** *473* (*1954*)). Much of the unsaturated fat of an animal cell resides in the mitochondria in close proximity to the cytochromes, and the latter contain some of the most potent catalysts of lipid peroxidation (Tappel, *Ibid.* **44,** *378* (*1953*)).

Thus in their present report, Zalkin and Tappel considered that the antioxidant effect of vitamin E could explain all the complicated metabolic functions of this vitamin.

Such studies may lead to a sufficient understanding of the action of vitamin E so that its place in clinical medicine may become firmly established.

NIACIN AND STEROL METABOLISM

High levels of niacin, niacinamide, isonicotinic acid and benzoic acid were fed to rats and chicks. Blood chloesterol levels in chicks were depressed by niacin, and in both rats and chicks by isonicotinic acid.

Niacin when fed in large doses to man effectively reduces plasma cholesterol levels in hypercholesterolemic patients, according to the report of W. B. Parsons, Jr., and J. H. Flinn (*Arch. Int. Med.* **103,** *783* (*1959*); *See also Nutrition Reviews* **17,** *78*, *168*, *291* (*1959*)). These authors found, however, that niacinamide does not lower blood cholesterol appreciably in man (*Circulation* **16,** *499* (*1957*)).

In order to investigate the mechanism by which blood cholesterol is reduced by niacin, J. L. Gaylor, R. W. F. Hardy and C. A. Baumann (*J. Nutrition* **70,** *292* (*1960*)) studied the effects of niacin and niacinamide as well as related compounds in rats and chicks. Blood and liver cholesterol were measured along with liver fat and pyridine nucleotides and fecal fatty acids, sterols and bile acids.

Male albino rats weighing 100 g. were fed diets containing from 0.1 to 1.0 per cent niacin, niacinamide or isonicotinic acid, and 1.0 per cent benzoic acid or 2 per cent tryptophan. The basal diet contained 18 per cent casein, 67 per cent sucrose, 10 per cent lard and adequate salts and vitamins. The chick basal diet contained in per cent: casein, 23; sucrose, 56; gelatin, 10; salts, 6; soybean oil, 4; and adequate vitamins. One per cent of niacin, niacinamide, isonicotinic acid or benzoic acid was added to the basal diet.

In the rat experiments, three groups of 12 rats were fed a diet containing 1 per cent cholesterol and 0.5 per cent cholic acid in order to elevate the blood cholesterol level, which increased from 100 mg. per cent to an average of 203 mg. per cent in two weeks. Then one group was continued on the original diet and the other two groups were given supplements of 0.2 and 1.0 per cent of niacin, respectively.

The cholesterol level rose steadily in all groups, but the rise was less rapid in the rats receiving the 1.0 per cent of niacin. Peak values at four weeks averaged 397 mg. per cent for controls, 340 mg. per cent for the 0.2 per cent niacin group and 450 mg. per cent for the group fed 1.0 per cent niacin. Group averages at seven weeks were 297, 306 and 292 mg. per cent. This indicates that, under these conditions, blood cholesterol in hypercholesterolemic rats does not respond to niacin therapy.

Since the above diet does not resemble the type of human diet in which niacin is usually effective, new groups of rats were given the basal diet containing 10 per cent lard as the only source of cholesterol.

Supplements were 0.1 and 1.0 per cent of niacin, niacinamide or isonicotinic acid, 2 per cent tryptophan and 1 per cent benzoic acid. The ad libitum consumption of the diet containing niacin resulted in significant increases in free and total cholesterol. Free cholesterol also increased with 2.0 per cent tryptophan feeding. Neither niacinamide nor benzoic acid changed the cholesterol level, but isonicotinic acid tended to reduce it.

In the chick experiments, both male and female chicks were fed ad libitum either the basal chick diet alone or supplemented with 10 per cent of lard or with lard plus 0.5 per cent cholesterol replacing the soybean oil and some sucrose. After two and four weeks, blood cholesterol was determined. The addition of cholesterol to the diet doubled the blood cholesterol level, the increase being mostly in ester cholesterol. After two weeks, the addition of 1 per cent niacin depressed the cholesterol significantly and the effect persisted at four weeks. Although niacin appeared to depress blood cholesterol in chicks fed lard with no added cholesterol, the differences were not significant. Only slight lowering was observed with niacinamide.

Blood cholesterol levels of chicks eating the basal soybean oil ration were significantly reduced by 1 per cent niacin or isonicotinic acid, but not significantly affected by niacinamide or benzoic acid.

Determination of pyridine nucleotide contents of livers showed a 100 per cent rise in rats and less in chicks upon feeding niacin, and a much greater increase when niacinamide was given. This suggests that the effectiveness of niacin in reducing blood cholesterol is not due to a greater conversion to pyridine nucleotides.

Liver fat and cholesterol were determined on rats and chicks fed the basal diet with and without niacin and related supplements. They were also measured following the feeding of rations containing lard or lard plus cholesterol and cholic acid, with and without niacin and related compounds. Cholesterol supplementation elevated the control liver cholesterol in rats from 3.8 mg. per g. of dry weight to over 21 mg. per g., and raised control values in the chick from 7 mg. per g. to over 26 mg. per g. Under these conditions, neither niacin nor niacinamide lowered liver cholesterol. In fact, substantial elevation occurred in the chicks.

Benzoic acid, niacin and niacinamide increased liver fat in the absence of added cholesterol in the rat, and isonicotinic acid lowered liver fat in both the rat and chick.

Liver cholesterol in chicks fed the ration with added cholesterol averaged 26.4 mg. per g. dry weight, and this was increased to 40.9 mg. per g. upon niacin supplementation and to 38.7 mg. per g. upon addition of niacinamide. The livers of rats and chicks fed the basal diets showed no change in liver fat or cholesterol after being fed niacin and related compounds.

In rats, a depressed growth rate followed the feeding of niacinamide, and niacinamide plus 2 per cent tryptophan reduced the growth rate to about 40 per cent of the control. However, neither niacin nor isonicotinic acid depressed growth. In chicks, growth was reduced by niacin from 8 to 17 per cent below that of control animals. A somewhat greater depression occurred with niacinamide and benzoic acid, while isonicotinic acid had no effect.

Measurement of fecal bile acids and fatty acids showed no alteration as a result of niacin feeding. Niacinamide increased the amount of fecal fatty acid and isonicotinic acid increased the bile acid excretion. Apparently the cholesterol-lowering effect of isonicotinic acid occurs in some metabolic event prior to and in preference to increased excretion of bile acids.

It is obvious from these studies that isonicotinic acid is by far the most con-

sistently effective and the least toxic of the drugs investigated.

Its effect on experimental lesions should certainly be studied. It seems likely that it may produce its effect by increasing the catabolism of cholesterol.

INHIBITION OF "BROWNING REACTION"

Inhibitors are described for the chromogenic reaction between glucose or fructose and fibrin hydrolysates. Fructose solutions with fibrin hydrolysates lose nutritive value less rapidly during storage than do glucose solutions.

Foodstuffs containing mixtures of amino acids and reducing sugars may deteriorate in quality because of the interactions of these two substances to form brown pigments. J. E. Hodge (*J. Agr. Food Chem.* **1,** *928 (1953)*) has described this "browning reaction" in detail. It has further been shown that the nutritive value of fibrin hydrolysates with dextrose is related inversely to the color of the solution (L. R. Overby and D. V. Frost, *J. Nutrition* **46,** *539 (1952)*). Subsequently, various sulfur-containing antioxidants were found to inhibit the development of color and delay loss of amino acid food value.

Recently, Overby, R. L. Fredrickson and Frost (*J. Nutrition* **69,** *318 (1959)*) compared potassium metabisulfite, sodium hydrosulfite and sodium formaldehyde sulfoxylate with respect to their capacity for inhibiting color formation in fibrin hydrolysate or glycine solutions with added glucose or fructose. Loss of nutritive value of the solutions was assayed by rat repletion.

The fibrin hydrolysate was a commercial preparation from beef fibrin containing about two-thirds free amino acids and one-third small peptides. Four solutions were prepared: (a) 80 g. of glycine and 200 of glucose diluted to 2000 ml. with water; (b) a similar solution of 80 g. of glycine and fructose; (c) 200 g. of glucose dissolved in a quantity of fibrin hydrolysate so that, when diluted to 2000 ml., the total nitrogen was 6.5 mg. per ml; and (d) a similar solution of 200 g. of fructose with fibrin hydrolysate.

The solutions were divided into 500 ml. portions and placed in closed bottles under carbon dioxide atmosphere. To three bottles of each of the four solutions one of the sulfur-containing inhibitors was added. All solutions were then autoclaved at 110° C for 20 minutes; a sample was then removed and the optical density (o.d.) measured at 385 mμ. The following optical densities were found in the four solutions autoclaved without inhibitors: fibrin hydrolysate with glucose, 1.8; fibrin hydrolysate with fructose, 0.830; glycine with glucose, 0.449; glycine with fructose, 0.790.

With fructose-fibrin hydrolysate, sodium formaldehyde sulfoxylate was the most effective inhibitor (o.d. = 0.323) followed by potassium metabisulfite (o.d. = 0.340) and sodium hydrosulfite (o.d. = 0.430). With fructose-glycine solutions, practically the reverse picture was observed; sodium formaldehyde sulfoxylate was least effective (o.d. = 0.251), sodium hydrosulfite was better (o.d. = 0.190), and potassium metabisulfite was best (o.d. = 0.035), but the degree of "browning" was less in all cases than with fibrin hydrolysate.

The order of effectiveness of the inhibitors with glucose-glycine and glucose-fibrin hydrolysate was not different from that found with fructose solutions, but all glucose-fibrin hydrolysate solutions showed more color and glucose-glycine solutions less color than the corresponding solutions with fructose.

For the nutritional studies, solutions of

fibrin hydrolysate with glucose or fructose were prepared as above with sodium formaldehyde sulfoxylate. Following sterilization at 110° C for ten minutes, they were either used immediately or further treated in one of the following ways before testing: (a) stored at 25° C for 15 months; (b) stored at 4, 25, and 40° C for four and a half months; (c) autoclaved 15 minutes at 121° C; and (d) autoclaved 30 minutes at 121° C.

Nutritive values of the "browned" solutions were determined by the rat repletion method of Frost and H. R. Sandy (*J. Biol. Chem.* **175,** *635 (1948)*). Rats on a diet of fibrin hydrolysate with 10 per cent glucose showed a gain of 6.1 g. in 12 days. Those on the 10 per cent fructose gained an average of 15.6 g. and those on a control solution of fibrin hydrolysate lacking carbohydrate gained an average of 19.8 g.

Fibrin hydrolysate with fructose after storage at 40° C for four and a half months showed about a 10 per cent loss in nutritive value by actual weight gain, but this was not statistically different from the weight gains of the other three groups. When an accelerated rate of browning was produced by heat treatment, it was found that 121° C for 15 minutes caused little or no loss of nutritive value, whereas 30 minutes exposure to this temperature resulted in a definite loss of nutritive value of the fibrin hydrolysate.

The authors point out that the three sulfite compounds employed in these experiments represent three different levels of oxidation-reduction potential. The least highly oxidized of these compounds, sodium formaldehyde sulfoxylate, was the most potent inhibitor in the case of the complex mixture of fibrin hydrolysate-carbohydrate. However, the model system glycine-carbohydrate was protected best by the most highly oxidized compound, bisulfite. No explanation for these seemingly contradictory results is offered.

CHOLESTEROL AND ESSENTIAL FATTY ACID DEFICIENCY

Cholesterol added to the ration of weanling male rats accelerated the development of essential fatty acid deficiency.

Attempts to accelerate the production of essential fatty acid deficiency led J. J. Peifer and R. T. Holman (*Arch. Biochem. Biophys.* **57,** *520 (1955)*) to try the stress of alloxan diabetes. They reasoned that since catabolism and mobilization of fat is accelerated in diabetes it could result in the concomitant raiding of essential fatty acid stores. Feeding weanling rats an essential fatty acid (EFA)-free diet and making them diabetic by injecting alloxan resulted in the development of severe EFA deficiency symptoms within one month.

Several facts suggest the possibility that cholesterol metabolism could be involved in the development of this EFA deficiency. Hypercholesteremia is associated with diabetes. Essential fatty acids are present in cholesterol esters and cholesterol esters tend to accumulate in the liver during the development of essential fatty acid deficiency. Thus the normal transport of cholesterol may depend on the presence of adequate supplies of EFA. For these reasons, Holman and Peifer (*J. Nutrition* **70,** *411 (1960)*) have studied the effects of feeding cholesterol along with hydrogenated coconut oil (HCO) on the deficiency of EFA.

Weanling male rats 21 days old were fed a fat-free diet for a week before being distributed at random into four groups eating diets with the following supplements: group one received 1 per cent hydrogenated coconut oil (HCO), group two received 1 per cent HCO and 1 per cent

cholesterol, group three received 1 per cent corn oil ethyl esters and group four received 1 per cent corn oil ethyl esters plus 1 per cent of cholesterol.

Within five weeks EFA deficiency appeared. The dermal score was more pronounced in animals receiving 1 per cent cholesterol. Growth was inhibited, food efficiency was diminished and the development of the testes was inhibited. Dietary cholesterol given to rats fed EFA rather than acting as a stress resulted in improved food efficiency, body weight gain and testes weight increase. Severe dermal symptoms normally appearing after three months of a fat-free diet regimen were manifest only five weeks following cholesterol supplementation.

Deficiency of essential fatty acids results in histological changes in rat testes, according to the report of T. C. Panos and J. C. Finerty (*J. Nutrition* **54,** *315* (*1954*)). If dietary cholesterol is used to hasten the depletion of essential fatty acids, severe degeneration occurs in spermatogenic tissue, according to Holman and E. Aaes-Jorgensen (*Proc. Soc. Exp. Biol. Med.* **93,** *175* (*1956*)). In the present experiment, rats fed an ESA-free diet had significantly lower testis weights than those fed EFA. Marked atrophy occurred in testes of some rats fed cholesterol and EFA-free diet.

Qualitative studies of essential fatty acid deficiency involved grading of the severity of dermal signs of deficiency. Groups of eight to ten 21-day-old weanling male rats were fed various combinations of diets with or without hydrogenated coconut oil (HCO), linoleate or cholesterol.

Group one fed a basal diet containing 1 per cent linoleate did not develop appreciable dermal signs. Groups two, three and four developed severe dermal signs after four weeks on a diet containing 1 per cent HCO and 1 per cent cholesterol. At the end of four weeks group two was fed 1 per cent linoleate without cholesterol and group three was fed 1 per cent linoleate with 1 per cent cholesterol, while groups one and four were continued on their original diets. The dermal symptoms of groups two and three improved equally, indicating the availability of EFA even in the presence of dietary cholesterol.

Group five fed 1 per cent cholesterol and 1 per cent linoleate did not show dermal signs of deficiency. Group six was fed 1 per cent HCO and 1 per cent cholesterol during the first four weeks. During the next three weeks cholesterol was removed but deficiency symptoms continued to become more severe. Groups seven and eight fed 1 per cent HCO plus 2 and 5 per cent cholesterol did not develop more severe symptoms than animals receiving less cholesterol, probably because these amounts exceeded the capacity of the rat intestine to absorb cholesterol as reported by T. M. Lin, E. Karvinin and A. C. Ivy (*Proc. Soc. Exp. Biol. Med.* **89,** *422*, (*1955*)).

During their studies of fatty acid deficiency, Holman and Peifer observed a great variation in the length of time needed to produce deficiency symptoms in 21-day-old weanling rats. Since the time varied from 20 to 60 days they were prompted to try the use of younger animals. After facing some problems of high mortality they were able to use 15-day-old rats in producing more severe EFA deficiency rapidly and uniformly.

Study of the polyenoic fatty acids of heart tissue was made in an effort to quantitate the effects of cholesterol on EFA deficiency. Various diets were fed the 15-day-old weanling male rats for 43 days. Analysis of the hearts showed that polyenes of EFA-deficient groups tended to accumulate in heart muscle, the largest accumulation being in the group fed 1 per cent HCO plus 1 per cent cholesterol. Trienes predominated while tetraenes represented a smaller proportion of total polyenes. Feeding of linoleate decreased the per cent of trienes and increased the per cent of tetraenes.

Total polyunsaturated fatty acids in-

creased in heart tissue of EFA-deficient rats. Since there are no dietary precursors, this increase probably represents mobilization rather than synthesis. The presence of extra cholesterol in the animal may result in an increased need for EFA in order to facilitate transfer of cholesterol.

Further work along this line may help to establish biochemical mechanisms by which relative EFA deficiency might lead to lipid accumulation in blood vessels. Whether this is a facet of lipoprotein structure and metabolism remains to be investigated. If cholesterol is transported and ultimately metabolised as a part of a lipoprotein complex, it may be that EFA may act mainly to regulate lipoprotein synthesis or catabolism.

HORMONAL FACTORS IN LIPID MOBILIZATION

Epinephrine and norepinephrine mobilize fat from adipose tissue in vitro. Purified corticotrophin does so without mediation of the adrenal glands.

Research on fat metabolism has excited much renewed interest since the demonstration by isotope techniques that fatty acids have an extremely rapid turnover in the body (*Nutrition Reviews* **17,** *241* (*1959*)) and that adipose tissue has a very active metabolism (*Ibid.* **17,** *280* (*1959*)). A number of physiologic substances, some long-known, affect the mobilization of depot fat. An earlier article on this subject (*Ibid.* **13,** *207* (*1955*)) discussed the role of local innervation of adipose tissue, C. H. Best's and J. Campbell's "adipokinin" from the anterior pituitary, and the specific pituitary and adrenal hormones. A more recent review discusses adrenals and fat mobilization (*Ibid.* **17,** *246* (*1959*)).

Suitable analytic techniques have permitted *in vitro* study of fragments of adipose tissue with measurement of various metabolites in the incubation medium under different conditions. "Lipolysis", or lipid mobilization from adipose tissue, now proves quite amenable to study and we are presented with a fascinating array of findings: epinephrine mobilizes lipid (see below), corticotrophin does so, but not through the adrenals (see below), and growth hormone has a similar effect. There is the lipid-mobilizing hormone of J. Seifter and D. H. Baeder, perhaps of pituitary origin (*Nutrition Reviews* **18,** *275* (*1960*)). D. Rudman's lipemia-producing hormone from the pituitary has quite different action (*Ibid.* **18,** *297* (*1960*)). Thus the pituitary may secrete several substances affecting mobilization of depot fat.

J. E. White and F. L. Engel (*Proc. Soc. Exp. Biol. Med.* **99,** *375* (*1958*)) studied the lipolytic action of epinephrine and norepinephrine on rat adipose tissue *in vitro*. They were led to this study because epinephrine injection in man causes an increase in serum nonesterified fatty acids. The promptness of the rise suggested that the effect was directly on adipose tissue; this had been shown to be true *in vitro* by the authors and others.

White and Engel incubated portions of epididymal adipose tissue from fasting rats in heparinized pooled rat plasma or serum at 36°C, with epinephrine or norepinephrine (solutions of L- or D- salts). Incubation for three hours without hormone caused no change in the fatty acid content of the medium and a decrease (due to artifact) in the fatty acid content of the tissue. However, 5 μg. of L-epinephrine caused a "striking" increase within three hours in the fatty acid content of the medium and in the adipose tissue. Exposure to 10 μg. of L-epinephrine for 15 minutes produced twice as much tissue fatty acid concentration as in tissue incubated without hormone; within

that time the plasma level did not change appreciably, suggesting that the new fatty acids were formed in the adipose tissue and not the plasma.

Incubation under nitrogen reduced considerably the epinephrine effect in medium and in tissue. The fatty acid content of the medium increased in the hormone-free controls under nitrogen, but not in air. White and Engel, therefore, infer that aerobic metabolism is required for the lipolytic action of epinephrine.

L-Epinephrine and L-norepinephrine were equally active and 100 to 200 times as potent as the respective D-isomers.

The authors conclude that epinephrine and norepinephrine probably stimulate hydrolysis of neutral fats directly in adipose tissue. The inactivity of the unphysiologic D-isomers strengthens their belief that the tissue response is a specific one.

Both corticotrophin and growth hormone produce fatty liver and ketonemia. White and Engel (*J. Clin. Invest.* **37,** *1556 (1958)*) found that they both had lipolytic effects *in vitro*.

These authors, in another paper entitled "Fat mobilization by purified corticotrophin in the mouse", have shown that this action is not adrenal-mediated (*Proc. Soc. Exp. Biol. Med.* **102,** *272 (1959)*). They removed one testis from etherized fasting mice and dissected away the epididymal fat pad. Hormones were injected intraperitoneally and the animals allowed to recover for six hours; water was allowed, but no food. The liver and second fat pad were then removed and the total extractable lipids in the two pads were compared. Mice adrenalectomized at the time the first fat pad was removed were maintained for the next six hours on 0.9 per cent saline solution.

Both growth hormone and corticotrophin (ACTH) caused a significant decrease in fat pad lipid (17.3 and 20.8 per cent respectively), but hydrocortisone did not. There was little change in liver fat. Fat pads of mice adrenalectomized when the first fat pad was removed showed fat loss (7.9 per cent). ACTH did not increase this significantly, and this weakens the authors' conclusions. However, when given together with hydrocortisone, it overcame the inhibition of lipolysis induced by the latter.

The authors infer, from these experiments and earlier *in vitro* work, that both growth hormone and corticotrophin act directly on adipose tissue depots. The lipolytic effect of growth hormone is much less than that of ACTH in equally ketogenic doses, and presumably not due to contamination. Although the importance of epinephrine and norepinephrine in this respect is not established, White and Engel infer that increased ACTH secretion secondary to adrenalectomy may account for the greater epididymal fat loss in adrenalectomized mice (7.9 per cent) than in the intact animals (2.5 per cent). They point out that hydrocortisone has no inhibitory effect *in vitro* and may well act *in vivo* by inhibiting ACTH secretion. The role of other pituitary hormones or of insulin was not studied.

Another of the accumulating reports on the *in vitro* endocrinology of adipose tissue is that of B. Leboeuf, R. B. Flinn and G. F. Cahill, Jr., entitled "Effect of epinephrine on glucose uptake and glycerol release by adipose tissue *in vitro*" (*Proc. Soc. Exp. Biol. Med.* **102,** *527 (1959)*).

These authors show that the release of fatty acids from adipose tissue is not necessarily linked to carbohydrate utilization. Although an inverse relationship obtains in many situations (*i.e.*, diabetes mellitus), they point out that epinephrine causes both glucose uptake and fatty acid release by adipose tissue *in vitro*.

Leboeuf, Flinn and Cahill incubated fragments of epididymal fat pads from fed rats in a buffer containing human serum albumin and glucose. The glucose, glycerol and fatty acid contents of the medium were determined after incubation for three hours with and without the addition of hormones.

Glucose uptake was increased by insulin and by epinephrine when compared to incubated controls. Free fatty acid was taken up slightly in the controls and with insulin but released by epinephrine. Likewise, glycerol release was slight in the controls, slightly less with insulin and considerable with epinephrine.

The authors point out that epinephrine inhibition of peripheral glucose utilization *in vivo* and in muscle incubated *in vitro* is probably attributable to glycogenolysis and that the virtual absence of glycogen from adipose tissue may account for the observed differences. They speculate as to why insulin and epinephrine have similar effects on glucose uptake but opposite effects on the release of free fatty acids, and conclude that epinephrine probably mobilizes fatty acids by increased glyceride breakdown rather than by decreasing carbohydrate availability.

The hormonal factors in lipid mobilization are evidently many, and their actions and interrelationships incompletely known. *In vitro* studies such as those reviewed above provide a means of extending our knowledge considerably.

VITAMIN A ACID AND THE FUNCTION OF VITAMIN A

Vitamin A acid maintains growth and general health of rats but cannot be stored and does not prevent night blindness or retinal changes.

In a recent study, J. E. Dowling and G. Wald, *Proc. Nat. Acad. Sci.* **44,** *648 (1958)*; *Nutrition Reviews* **17,** *22 (1959)*) investigated the course of vitamin A deficiency in rats. From the results, the authors concluded that vitamin A (as the aldehyde, retinene) is not only involved with prevention of night blindness but also with protection of the protein, opsin, and the rods, which deteriorate in severe vitamin A deficiency. Indeed, it was suggested that the general effect of this vitamin, the lack of which results in the disintegration of epithelial and other tissues, might lie in a similar protection of structural proteins in these tissues by combination with some derivative of vitamin A.

Continuing the study of this exciting possibility, Dowling and Wald (*Proc. Nat. Acad. Sci.* **46,** *587 (1960)*) investigated the effect of vitamin A acid on vitamin A-deficient rats. This acid, which is identical in structure to vitamin A except for the oxidation of the primary alcohol of the latter to a carboxyl, had previously been shown by J. F. Arens and D. A. Van Dorp (*Nature* **158,** *622 (1946)*) to prevent obvious signs of vitamin A deficiency without contributing any vitamin A to liver storage.

Several different types of experiments were performed:

To test the effect of vitamin A acid on growth and general condition, weanling rats were raised on an otnerwise adequate vitamin A-deficient diet with or without supplementation with vitamin A acid (at 50 μg. per day) or vitamin A alcohol. The animals without the supplements grew normally for about 40 days, then stopped growing and died at about 60 days. Those with the vitamin A acid supplement grew about as rapidly as did those with vitamin A alcohol and, except for visual difficulties, appeared perfectly normal to external examination. Moreover, the acid was immediately effective in restoring growth of depleted animals. It was evident, therefore, that for growth and general health the acid was as effective as the alcohol.

To investigate the possible conversion of vitamin A acid to the alcohol, liver stores of rats maintained on the acid were analyzed. It was found that liver vitamin A declined as rapidly in the animals with vitamin A acid supplement as in those on the deficient diet alone. Moreover, there is apparently

no storage of the acid since, when the acid supplement was removed, rats apparently in good condition stopped growing and became severely deficient in a few days. In normally nourished adult animals, large stores of vitamin A usually maintain the animals for long periods after removal of the vitamin from the diet. Sensitive chemical tests for vitamin A acid also failed to reveal the presence of any of the acid in livers of rats receiving this supplement and thus confirmed the results of the growth studies.

It is evident, then, that the vitamin A acid is very rapidly destroyed and that it cannot be reduced to the aldehyde or alcohol.

The results of a study of the visual thresholds of rats on the acid-supplemented diet are of particular interest. In the unsupplemented rats dying at about 60 days, moderately severe night blindness had developed with visual thresholds about 100 times normal (the visual threshold is the smallest luminance of a $\frac{1}{50}$ second flash light needed to evoke a just perceptible electroretinogram (ERG)). The rats with the vitamin A acid supplement developed night blindness at the same rate but since they were otherwise in good condition, continued to show higher visual thresholds until at about 140 days they had leveled off at about 2000 to 3000 times normal. At this time, the retinal rhodopsin was found to be only 1 to 5 per cent of normal. When the diet was continued, it was found that visual stimuli even 10,000 times the threshold level failed to yield a normal ERG response, and after ten months the animals were completely blind.

Examination of the retinas of the deficient animals with the electron microscope revealed that, as the visual threshold increased, the visual cells decreased and the outer segments of the rods began to disintegrate. After ten months, only a single row of nuclei remained of the visual cells and both inner and outer segments of the rods had disappeared.

When vitamin A alcohol was administered to animals that had been on the acid supplementation for different lengths of time, varying degrees of recovery were obtained. Up to ten weeks, recovery was rapid and complete. At 25 weeks, a two phase recovery was seen: a rapid recovery to visual thresholds about ten times normal and a much slower return to normal. The authors concluded that the first stage represents combination of retinene with the opsin of the remaining rods, while the slower stage was for resynthesis of opsin and reformation of lost rods.

After ten months, recovery could not be elicited and the visual cells had apparently been lost or had dedifferentiated to the extent that they could no longer form outer segments.

The authors have come to several significant conclusions:

First, vitamin A alcohol (the transport form of the vitamin and the storage form, as the ester) is oxidized to retinene in the retina, in spite of an unfavorable equilibrium, because of the rapid removal of the aldehyde in its combination with opsin to form rhodopsin. This is apparently the only function of the aldehyde. The alcohol is also oxidized to the acid, presumably in the liver, and the latter is either rapidly converted to an active product or is rapidly used in its functions of growth and tissue maintenance. In any event, the acid (or its product) may be the important form of vitamin A in its general function, but it must be stored as the alcohol and cannot be reconverted to either the aldehyde or alcohol.

With these experiments, another step has been taken in the understanding of the functions of vitamin A. It is to be hoped that information on the exact mechanism by which vitamin A acid may act in maintaining epithelial tissue will be shortly forthcoming.

NOTES

Letters to the Editor

Sir:

In a special article (*Nutrition Reviews* **15,** *257 (1957)*) D. M. Hegsted indicated the need for a re-examination of the situation concerning the present high recommended intakes of calcium. Supporting his view (*Ibid.* **16,** *31 (1958)*), I questioned the wisdom of recommending luxus intakes of the element in the absence of specific deficiency stigmata.

In a recent study on pathological grading and chemical composition of aorta from Bantu and white subjects, we drew attention to the far higher concentration of calcium in white compared with Bantu aorta from middle age onwards, and even in aorta of equal pathological grading (M. Anderson *et al., A. M. A. Arch. Path.* **68,** *380 (1959)*). In a paper just published, A. E. Hirst Jr., *et al.* (*Arch. Path.* **69,** *578 (1960)*) also have noted the far greater incidence and severity of calcification lesions in aorta from Los Angeles whites compared to Indians in South India. Both the latter population and the South African Bantu have low calcium intakes, lesser severity of aortic and coronary lesions, and a much lower mortality from coronary heart disease.

The precise bearing of arterial calcification on mortality from coronary heart disease is not known. But if, to ward off death from this cause, a reduction in the amount of fat consumed is recommended, would it not be logical also to recommend a modicum of reduction in the present high calcium intakes ingested by more privileged populations?

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Dear Sir:

The review of the effect of antioxidant on vitamin E deficiency in chicks (*Nutrition Reviews* **17,** *340 (1959)*) prompts the following comments which may be useful, since much of our work was not submitted to journals normally read by many investigators in animal nutrition.

In all species tested to date (man: *Am. J. Clin. Nutrition* **4,** *408 (1956)*; **8,** *451 (1960)*; *A. M. A. Arch. Neurol.* **1,** *312 (1959)*; the chick: *Proc. Soc. Exp. Biol. Med.* **102,** *375 (1959)*; *A. M. A. Arch Gen Psychiat.* **1,** *420 (1959)*; the rat: *J. Nutrition* **72,** *Nov. issue (1960)*), the level of alpha-tocopherol required to inhibit reactions attributable to vitamin E deficiency were functions of the amounts of peroxidizable compounds present in the diet or in the tissues. For example, little if any added tocopherol is required when only small amounts of polyunsaturated fatty acids are present, but the requirement for tocopherol increases markedly as the level of linoleic acid in the diet is increased.

Accordingly, one should never interpret an experiment on tocopherol need without evaluating how much total polyunsaturated fatty acid is in the diet. It should be emphasized that even lard, which normally contains about 10 per cent linoleic acid (and much more if the pig was fed unsaturated fats), can provide as much linoleic acid at a 20 per cent level in the diet as corn oil at a 4 per cent level. Both of these levels of fat are sufficient to produce encephalomalacia in most chicks deprived of tocopherol since they provide more than 2 per cent linoleic acid in the diet.

The fatty acid profile of all of the tissue lipoproteins and phospholipids tested today are influenced by the levels of various fatty acids in the lipids consumed, either from the diet or as a result of previous ingestion. The effect of diet on tissue lipid composition, which hog raisers have con-

sidered to be important for many years, is just as important in determining the fatty acid composition of brain mitochondria and erythrocytes (*Science* **130,** *917 (1959)*). The types of pathology noted during vitamin E deficiency may be related to the rates of growth (lipid accumulation), the relative alterations in the polyunsaturated fatty acid composition of the organs affected, and whether or not there is adequate antioxidant at the site of the enzymes or other microconstituents of the cells in question.

The means by which changes in fatty acid composition can affect physiological function is not yet clear, but a recent report by F. D. Collins (*Nature* **186,** *366 (1960)*) showing that the more unsaturated lecithins from rat livers took up more P^{32} should be considered in the interpretation of these effects.

Presumably, other lipid-soluble antioxidants could function as well as alpha-tocopherol if they were completely nontoxic and if they could be absorbed and directed to the specific intracellular sites that need protection, but to date no complete substitute for alpha-tocopherol has been found. Since the cellular absorption and excretion rates of synthetic antioxidants can be expected to be different from those of alpha-tocopherol, one should not be surprised to obtain different physiological effects when they are used. Such differences do not prove that vitamin E has additional functions other than that of an antioxidant.

Although some chemical signs of lipid peroxidation, such as increases in "ceroid" pigment, are not difficult to demonstrate either as a function of acute vitamin E deficiency or as a consequence of long exposure to accumulated oxidations, as in aged animals, the demonstration of specific peroxidized lipids in tissue has not been entirely satisfactory. Unfortunately, the thiobarbituric acid test for peroxides is in part a function of the amount of tocopherol and the amounts of peroxidizable lipids present in tissue, and the technique as it is usually conducted measures amounts of peroxide formed during the procedure as well as the amounts of preformed peroxide products. More work needs to be done to prove that peroxides are formed in tissue since it is logical that they should be present during vitamin E deficiency.

Vitamin E may have antioxidant functions other than that of protecting polyunsaturated fatty acids. Thus it may be useful in protecting vitamin A, certain sterols and other tissue constituents not now recognized. In the above discussion, selenium is considered to be supplied adequately and is not a part of this antioxidant story, but the possibility remains that on certain special diets there is need for antioxidant protection of some unknown vital selenium compound or of other metallo-organic complexes.

M. K. Horwitt
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Recent Books

Food for Space Travel. By Albert A. Taylor, Beatrice Finkelstein and Robert E. Hayes. Air Research and Development Command Technical Report No. 60-8, Andrews Air Force Base. Copies may be obtained from the Office of Technical Services, U. S. Department of Commerce, Washington 25, D. C. Pp. 64.

Manual of Nutrition, Fifth Edition. Prepared by the Ministry of Agriculture, Fisheries and Food and the Central Office of Information. Published by Her Majesty's Stationery Office, London. 1959. Pp. 72. Price 3 shillings net.

Erratum

The italicized summary of the review "Scurvy and Blood Coagulation" appearing on page 242 of the August 1960 issue should have stated that ascorbic acid deficiency resulted in an "increase in platelets" rather than a "reduction".

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